Journal of Medical Virology 51:119–125 (1997)

Detection of HPV DNA in Trichilemmomas by Polymerase Chain Reaction

Angela Rohwedder[1]*, Oliver Keminer[1], Carlo Hendricks[2], and Jörg Schaller[2]

[1]*Department of Medical Microbiology and Virology, Ruhr-University Bochum, Germany*
[2]*Department of Dermatology, Dermatohistological Unit, St. Barbara Hospital Duisburg, Germany*

Paraffin sections of 11 formalin-fixed trichilemmomas were investigated for the presence of human papillomavirus (HPV) DNA by the polymerase chain reaction (PCR) with the degenerated consensus primer pairs. PCRs were conducted with different annealing temperatures. When the annealing temperature was reduced from 55°C to 50°C, amplification products of the expected size were obtained for all 11 cases investigated. Determination of the HPV type was performed by cloning and sequencing of the amplification products.

The sequence analysis of the eleven cloned amplicons gave the following data: based on sequence comparison with published amino acid sequences, the best homology was found to epidermodysplasia verruciformis (EV)-associated HPVs (supergroup B). In four specimens an HPV type 23 related type was found; five specimens contained HPV sequences which did not match with one of the known HPV types, but had the closest homology to HPV types 15, 17, and 37. Three of the HPV variants which had not been characterised, displayed identical sequences. Two additional HPV amplification fragments displayed 100% homology to HPV-6b.

These results demonstrate, for the first time, the presence of HPV DNA in trichilemmomas. The sequence data suggest that HPV variants or types in trichilemmoma are members of the EV-associated HPV supergroup B. ***J Med Virol 51:119–125, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: human papillomavirus; polymerase chain reaction; epidermodysplasia verruciformis-associated HPVs

INTRODUCTION

Human papillomaviruses (HPV) are capable of inducing benign and malign skin tumours by infecting epithelial cells. Today more than 70 HPV genotypes are identified and can be categorised into five supergroups A–E: the genital HPVs (A); the epidermodysplasia verruciformis (EV)-associated HPVs (B); ungulate associated papillomaviruses (C and D) and cutaneous HPVs (E) [Chan et al., 1995]. Cross-hybridisation studies have shown that the EV-associated HPV supergroup B can be divided into several subgroups of related genotypes [deVilliers 1989; Kremsdorf et al., 1984]. The EV-associated HPVs were found, in particular, on human skin, in typical warts and squamous cell carcinomas.

The broad spectrum of epithelial hyperplasias which is induced by HPV displays a unique morphology with papillomatosis and acanthosis, hypergranulosis and vacuolated keratinocytes. Such morphological criteria are found mainly in fresh verrucous lesions of the skin, whereas older lesions have little or no koilocytes. The architecture of the viral wart is, however, still present.

Trichilemmoma, first described by Headington and French in 1962 as benign neoplasm of the hair follicle, exhibit many features of an advanced viral wart. These tumours are non-specific clinically and generally appear as small, solitary, verrucous, domed, or smooth-surfaced papules on the face and neck. Multiple trichilemmomas are a hallmark of Cowden's disease, a rare genodermatosis in which malignancies, mainly of the breast and the thyroid, may develop.

Histologically, trichilemmomas are characterised as sharply circumscribed, lobular epithelial proliferations in continuity with the epidermis, which exhibit a prominent, clear cell component. Other features are peripheral nuclear palisading, a thickened basement membrane, and varying degrees of hyperkeratosis and parakeratosis [Ackermann, 1980]. Central follicular structures are often present.

Whether trichilemmomas are true benign neoplasms or virus-induced hyperplasias remains a current subject of debate. Earlier studies using immunoperoxidase techniques did not reveal any evidence for papillomavirus infection [Penneys et al., 1984]. In the first study

*Correspondence to: Angela Rohwedder, Department of Medical Microbiology and Virology, Ruhr-University Bochum, Universitatsstrasse 150, 44801 Bochum, Germany.

Accepted 11 August 1996

© 1997 WILEY-LISS, INC.

on HPV DNA in trichilemmomas, by means of the polymerase chain reaction, no specific amplification product could be detected [Leonardi et al., 1990]. In view of the fact that human papillomaviruses are a virus group with high sequence heterology, detection of HPV DNA, depends, perhaps, on the use of broad spectrum primers and well established PCR conditions. The occurrence of HPV types in premalignant and malignant skin lesions of renal transplant recipients [Dyall-Smith et al., 1991; Tieben et al., 1993] ranged from low to nonexistent. In contrast, Berkhout and co-workers [1995] detected EV-associated HPV types and untypable new variants in cutaneous cancers from renal transplant recipients by using a nested PCR assay with a new set of degenerated primer pairs.

We investigated tissue sections of 11 paraffin-embedded trichilemmomas by polymerase chain reaction (PCR), using different PCR conditions and two broad-spectrum consensus primer sets [MY 11/09 described by Manos et al., (1989) and the primer sets described by Berkhout et al., (1995)].

MATERIALS AND METHODS

Trichilemmomas

Eleven cases of typical trichilemmomas (Fig. 1), characterised by the morphological criteria of Headington and French [1962], were selected from the Dermatopathology files of the Department of Dermatology, St. Barbara Hospital Duisburg, Germany. Tissues were formalin-fixed and paraffin embedded.

DNA-Extraction

Five sections of each paraffin-embedded tissue sample were deparaffinized and DNA was extracted using the QIAamp tissue kit (QIAGEN, Germany). The DNA of each tissue sample (five sections of each sample) was eluted in 100 µl water, divided into 50 µl aliquots, and stored at −20°C until use.

Extracted DNA of the tissue samples were confirmed by using β-globin primer [Saiki et al., 1988]. To exclude contaminations negative controls (water only and HPV negative tissue samples) were included. Cross-contaminations between samples and positive controls could be ruled out, because only positive controls for HPV-5, -16, and -18 were available [Schaller et al., 1996].

Primer

Polymerase chain reactions were carried out with the primer pair MY 11/MY 09 which direct the amplification of a 450 bp fragment of the HPV L1 open reading frame of many HPV types [Ting and Manos, 1989] and with the primer pairs CP65/70 and CP66/69 [Berkhout et al., 1995].

Polymerase Chain Reaction (PCR)

Polymerase chain reactions were conducted in 100 µl reaction volumes. Each reaction contained 50 mM KCl, 20 mM Tris-HCl (pH 8,4), 1,5 mM $MgCl_2$, 0,2 mM of each deoxynucleotide triphosphate, 20 pmol of each primer, 2 U Taq DNA-polymerase (Gibco/BRL, Eggenstein, Germany), and 5 µl template-DNA. The reaction mixture was overlaid with mineral oil and subjected to 40 amplification cycles. The cycle profile consisted of 2 minutes at 72°C (synthesis), 1 minute at 55–50°C for primer pair MY 11/09 or 1 minute at 50°C for the primer sets CP65/70 and CP66/69 (primer annealing), and 95°C for 1 minute (denaturation). For each trichilemmoma two independent PCRs were conducted with the primer pair MY 11/09 at the different annealing temperatures. In view of the very high risk of contamination the second PCRs were carried out by another person and in a different PCR laboratory. Amplification with MY 11/09 produce a DNA fragment of 450 bp, and CP66/69 produce an amplicon of 452–467 bp. Amplification products were analysed by electrophoresis on 2% agarose gels.

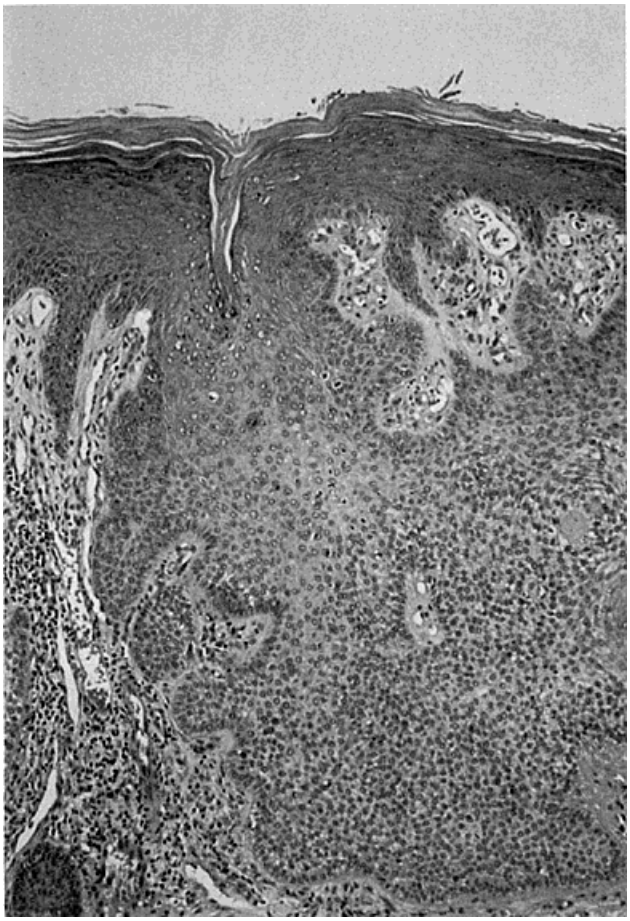


Fig. 1. Paraffin-embedded section of trichilemmoma HE ×100.

Isolation and Cloning of the Amplification Products

Amplification products of the expected size were recovered from agarose gels by using the gene clean II kit (Dianova, Germany) and then ligated to the pUC57/T cloning vector (Fermentas, Germany). This vector allows the direct cloning of PCR-products, as described by Marchuk et al. [1990]. After the transformation of

competent bacteria (strain RRI), positive transformants were identified by restriction analysis of small-scale preparations of plasmid DNA [Sambrook et al., 1992]. To exclude mismatches caused by the Taq polymerase, the amplification product of two independent amplification reactions were cloned and sequenced for each sample.

Sequencing

Sequencing of cloned PCR-fragments was achieved by cycle sequencing with "Prism TM Ready Reaction Dye DeoxyTM Terminator Cycle Sequencing Kit" (Applied Biosystems, Weiterstadt, Germany) and using the universal and reverse M13 primer (Pharmacia, Freiburg, Germany) as sequencing primer. The sequencing reactions were analysed on a 373 DNA sequencer (Applied Biosystems).

Sequence Analysis

Sequence alignment and calculation of the distances between the HPV types were done by using the CLUSTAL W computer program.

RESULTS

Total DNA was isolated from paraffin-embedded skin sections of 11 trichilemmomas and further analysed by polymerase chain reaction with different primer sets and different PCR conditions. No amplification products were obtained with the primer pairs CP65/70 and CP66/69 (Fig. 2a). Using the common consensus primer pair MY 11/09 and 55°C annealing temperature, we detected faint DNA bands of the correct molecular weight for only two samples (Fig. 2b, lanes 2 and 3).

In view of the fact that the annealing temperature for this primer pair may be too high for detection of the broad spectrum of EV-associated HPV types and variants, we reduced the temperature to 50°C. As shown in Figure 2c, amplification products of 450 bp were detected in all samples. In addition to the expected amplicon, one or two unspecific products were amplified in five samples. In order to confirm that these bands represent nonspecific HPV-PCR products, the fragments were isolated, cloned into pUC57/T, and, subsequently, sequenced. No significant homology between these sequences and HPV sequences were observed.

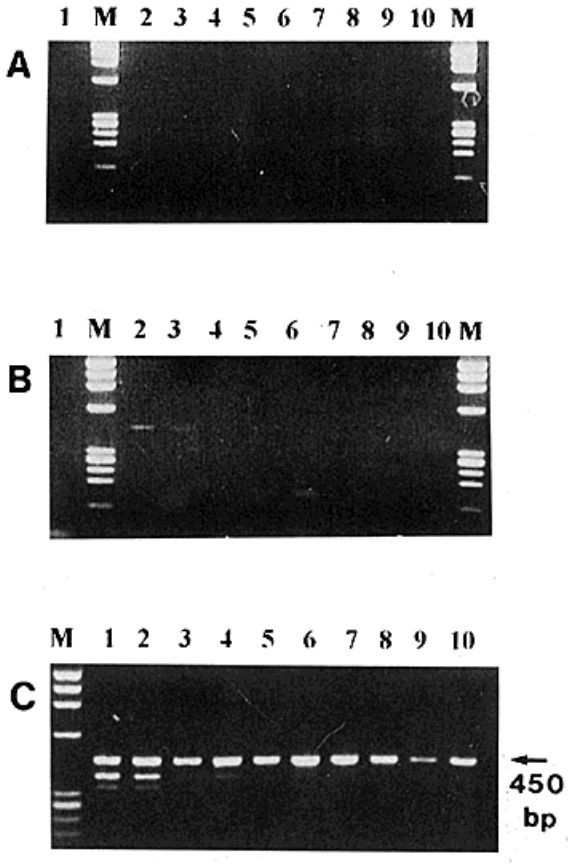


Fig. 2. Agarose gel electrophoresis of the PCR products. **A:** Result of the amplification reactions of 10 cases with the primer pair CP70/65. **B:** Result of the amplification reactions of the same probes as in A but with primer pair MY 11/09 using an annealing temperature of 55°C. Only in lane 2 and 3 a faint DNA band of the correct size is seen. **C:** Result of the amplification reactions of the same probes as in A and B but with primer pair MY 11/09 using an annealing temperature of 50°C. A specific PCR product of 450 bp is seen in every lane. Additional unspecific products are in lane 1, 2, 3, 4, and 6. Lane M: Molecular weight marker DNA Phi-X-Hae III digested.

Sequence Analysis of the HPV PCR Fragments

Comparison of the deduced amono acid sequences showed that the 11 sequenced MY 11/09 fragments belonged to five different HPV sequences: HPV-23TRI, HPV-X, HPV-Y, HPV-Z, and HPV-6. The deduced amino acid sequences of the five sequences are listed in Figure 3. Comparison with sequences of known HPV types revealed that nine of the eleven sequenced fragments belonged to the epidermodysplasia verruciformis-associated supergroup B, described by Chan and co-workers [1995], and two sequenced fragments belonged to HPV type 6.

The finding of HPV type 6 in two cases is unexpected, but not unusual, since HPV 6 was also found in oral and laryngeal mucosas and in cutaneous epithelia. We cannot exclude the presence of more than one HPV type in some cases, since they may not be detectable by the employed procedure. Even when sequencing two independent clones, one relies on statistical probability to identify a mixed infection. For the positive detection of possible multiple infections it is necessary to screen all clones by hybridization or restriction analysis.

Figure 4 shows a multiple alignment of the detected new sequences and all known EV-associated HPV types. The multiple alignment was constructed according to the alignment described by Berkhout et al., [1995]. The greatest differences were observed in the 5′end part of the sequences which represent a stretch of great sequence diversity, with extensive insertions and/or deletions. On the other hand, the 3′end part, ranging from amino acid (aa) 26 to aa 92 (Fig. 4), represents a very conserved amino acid stretch. Comparing only the conserved stretches of the aligned sequences, we found that

```
HPV-TYPE X     GILWGNQMFI TVADNTRNTN FTISVTSDGS TITEYNTQNI R---EFLRHVEEY  50
HPV-TYPE Y     GILWGNQMFI TVADNTRNTN FSISVTTEAG A-TEYNSQNI R---EFLRHVEEY
HPV-TYPE Z     GILWGNQLFV TVLDNTRNTN FSIAVHQEQK QVKEIQNYDS AKFNEFQRHVEEY
HPV-TYPE 23TR GILWNNQIFV TVADNTRNTN FSISVKSEDS LAN-YNASNI R---EYMRHVEEY
HPV-TYPE 6     GICWGNQLFV TVVDTTRSTN MTLCASVTTS STY-TNS-DY ---KEYMRHVEEY

HPV-TYPE X     QLAIILQLCK VPLQAEVLTQ INAMNAGILE EWQLGFVPTP DNAVHDIYRY    100
HPV-TYPE Y     QLSLILQLCK VPLKAEVLTQ INAMNSGILE EWQLGFVPTP DNSVHDTYRY
HPV-TYPE Z     EVSLILQLCK IPLKAEVLAQ INAMNSDILE NWQLGFVPTP DNPIHDTYRY
HPV-TYPE 23TR QLSFILQLCR IPLKAEVLTQ INAMNSDILE NWQLGFVPTP DNAVHDTYRY
HPV-TYPE 6     DLQFIFQLCS ITLSAEIMAY IHTMNPSVLE DWNFGLSPPP NGTLEDTYRY

HPV-TYPE X     IDSKATKCPD AVAPKDKEDP FGKYTFWNVD LTEKLSLDLD QFPLG         145
HPV-TYPE Y     INSKATKCPD AVVPKEKEDP FAKYSFWHVD LTEKLSLDLD QYPLG
HPV-TYPE Z     LDSLATRCPE KVPAKEKVDP YAKYVFWNVD LSERLSLDLD QFPLG
HPV-TYPE 23TR LASKATKCPD AVPETQKEDP FGKYSFWNVD MKEKLSLDLD QFPLG
HPV-TYPE 6     VQSQAITCQK PTPEKEKPDP YKNLSFWEVN LKEKFSSELD QYPLG
```

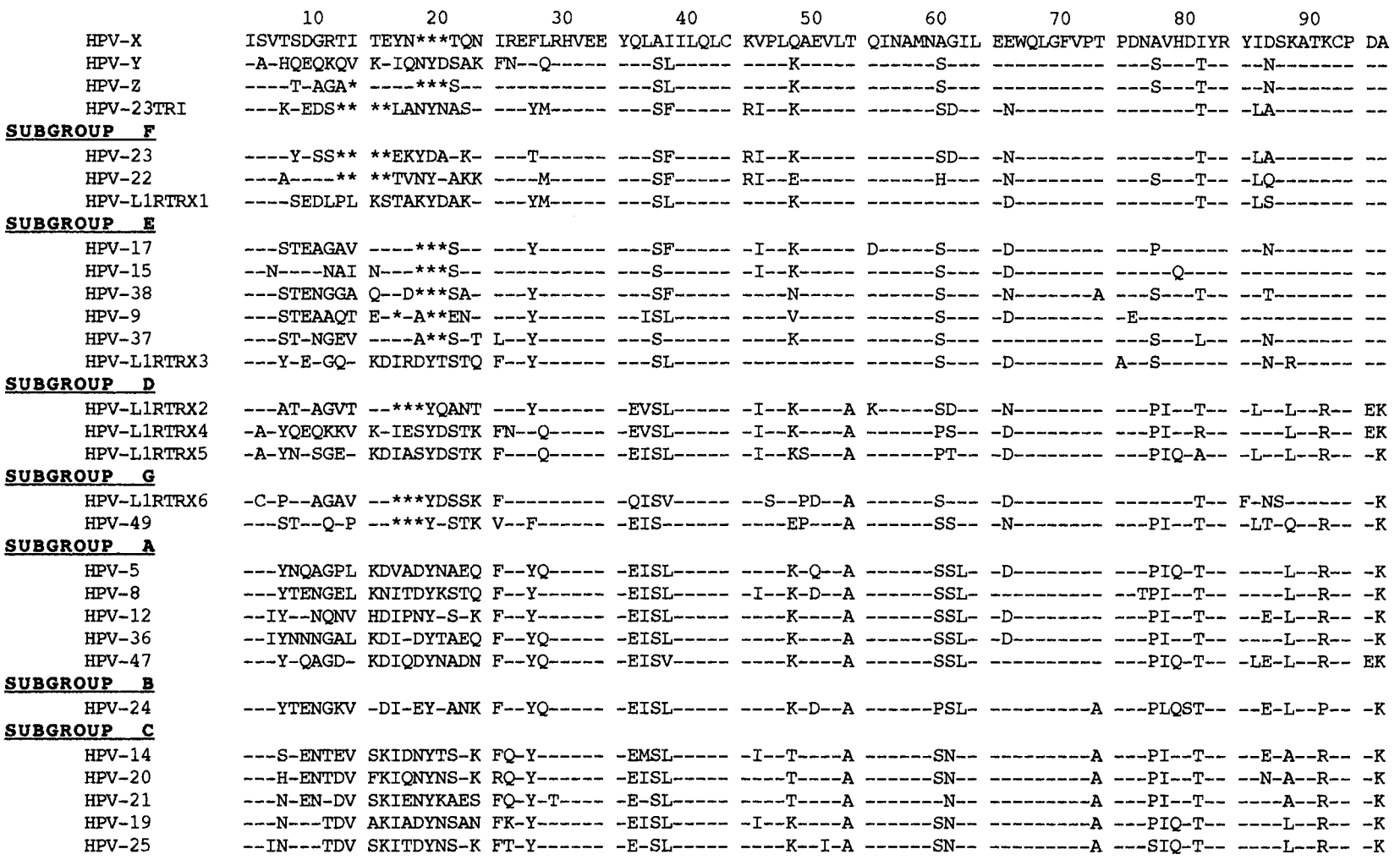
Fig. 3. Deduced amino acid sequences of the cloned trichilemmomas HPV PCR fragments; (−) sequence shift to get the best fit alignment.

HPV-23 and the sequence HPV-23TRI, which was found in four specimens, were 100% identical. The sequence HPV-X, detected in three samples, had the closest homology to HPV type 15 or 37 (91%). The sequences HPV-Y and HPV-Z, which differ only in the 5′end variable stretch, were closely related to HPV-37. Only two amino acid exchanges, corresponding to 97% homology, were found between HPV-37 and HPV-Y/Z. HPV-Y as well as HPV-Z was detected in only one specimen. The amino acid diversity to the most closely related EV-associated HPV types is summarized in Table 1.

Despite the very close relationship to the EV HPV types 23, 15, and 27, which was demonstrated, the numerous amino acid changes within the highly variable region of the amplified MY 11/09 fragment indicate that all detected EV-associated HPV sequences presumably represent putatively novel HPV types.

DISCUSSION

Using the degenerate consensus primer pair MY 11/09 and modified amplification conditions we have detected, for the first time, HPV DNA in 11 trichilemmomas. Our detection of HPV sequences in trichilemmomas contrasts sharply to the only other investigation of HPV DNA in trichilemmoma, whereby all cases are negative [Leonardi et al., 1990]. Leonardi and co-workers used an annealing temperature of 55°C for the primer pair MY 11/09, as described by Manos et al.



```
                    10         20         30         40         50         60         70         80         90
HPV-X               ISVTSDGRTI TEYN***TQN IREFLRHVEE YQLAIILQLC KVPLQAEVLT QINAMNAGIL EEWQLGFVPT PDNAVHDIYR YIDSKATKCP DA
HPV-Y               -A-HQEQKQV K-IQNYDSAK FN--Q----- ---SL----- ----K----- ------S--- ---------- ---S---T-- --N------- --
HPV-Z               ----T-AGA* ----***S-- ---------- ---SL----- ----K----- ------S--- ---------- ---S---T-- --N------- --
HPV-23TRI           ---K-EDS** **LANYNAS- ---YM----- ---SF----- RI--K----- ------SD-- -N-------- -------T-- -LA------- --
SUBGROUP F
HPV-23              ----Y-SS** **EKYDA-K- ---T------ ---SF----- RI--K----- ------SD-- -N-------- -------T-- -LA------- --
HPV-22              ---A-----** **TVNY-AKK ----M----- ---SF----- RI--E----- ------H--- -N-------- ---S---T-- -LQ------- --
HPV-L1RTRX1         ----SEDLPL KSTAKYDAK- ---YM----- ---SL----- ----K----- ---------- -D-------- -------T-- -LS------- --
SUBGROUP E
HPV-17              ---STEAGAV ----***S-- ---Y------ ---SF----- -I--K----- D-----S--- -D-------- ---P------ --N------- --
HPV-15              --N----NAI N---***S-- ---------- ---S------ -I--K----- ------S--- -D-------- -----Q---- ---------- --
HPV-38              ---STENGGA Q--D***SA- ---Y------ ---SF----- ----N----- ------S--- -N-------A ---S---T-- --T------- --
HPV-9               ---STEAAQT E-*-A**EN- ---Y------ --ISL----- ----V----- ------S--- -D-------- -E-------- ---------- --
HPV-37              ---ST-NGEV ----A**S-T L--Y------ ---S------ ----K----- ------S--- ---------- ---S---L-- --N------- --
HPV-L1RTRX3         ---Y-E-GQ- KDIRDYTSTQ F--Y------ ---SL----- ---------- ------S--- -D-------- A--S------ --N-R----- --
SUBGROUP D
HPV-L1RTRX2         ---AT-AGVT --***YQANT ---Y------ -EVSL----- -I--K----A K-----SD-- -N-------- ---PI--T-- -L--L--R-- EK
HPV-L1RTRX4         -A-YQEQKKV K-IESYDSTK FN--Q----- -EVSL----- -I--K----A ------PS-- -D-------- ---PI--R-- ----L--R-- EK
HPV-L1RTRX5         -A-YN-SGE- KDIASYDSTK F---Q----- -EISL----- -I--KS---A ------PT-- -D-------- ---PIQ-A-- -L--L--R-- -K
SUBGROUP G
HPV-L1RTRX6         -C-P--AGAV --***YDSSK F--------- -QISV----- --S--PD--A ------S--- -D-------- -------T-- F-NS------ -K
HPV-49              ---ST--Q-P --***Y-STK V--F------ -EIS------ ----EP---A ------SS-- -N-------- ---PI--T-- -LT-Q--R-- -K
SUBGROUP A
HPV-5               ---YNQAGPL KDVADYNAEQ F--YQ----- -EISL----- ----K-Q--A ------SSL- -D-------- ---PIQ-T-- ----L--R-- -K
HPV-8               ---YTENGEL KNITDYKSTQ F--Y------ -EISL----- -I--K-D--A ------SSL- ---------- --TPI--T-- ----L--R-- -K
HPV-12              --IY--NQNV HDIPNY-S-K F--Y------ -EISL----- ----K----A ------SSL- -D-------- ---PI--T-- --E-L--R-- -K
HPV-36              --IYNNNGAL KDI-DYTAEQ F--YQ----- -EISL----- ----K----A ------SSL- -D-------- ---PI--T-- ----L--R-- -K
HPV-47              ---Y-QAGD- KDIQDYNADN F--YQ----- -EISV----- ----K----A ------SSL- ---------- ---PIQ-T-- -LE-L--R-- EK
SUBGROUP B
HPV-24              ---YTENGKV -DI-EY-ANK F--YQ----- -EISL----- ----K-D--A ------PSL- ---------A ---PLQST-- --E-L--P-- -K
SUBGROUP C
HPV-14              ---S-ENTEV SKIDNYTS-K FQ-Y------ -EMSL----- -I--T----A ------SN-- ---------A ---PI--T-- --E-A--R-- -K
HPV-20              ---H-ENTDV FKIQNYNS-K RQ-Y------ -EISL----- ----T----A ------SN-- ---------A ---PI--T-- --N-A--R-- -K
HPV-21              ---N-EN-DV SKIENYKAES FQ-Y-T---- -E-SL----- ----T----A -------N-- ---------A ---PI--T-- ----A--R-- -K
HPV-19              ---N---TDV AKIADYNSAN FK-Y------ -EISL----- -I--K----A ------SN-- ---------A ---PIQ-T-- ----L--R-- -K
HPV-25              --IN---TDV SKITDYNS-K FT-Y------ -E-SL----- ----K--I-A ------SN-- ---------A ---SIQ-T-- ----L--R-- -K
```

Fig. 4. Amino acid comparison of the trichilemmoma HPV types to known EV-associated HPV types (subgroup A–G). Multiple sequence alignment of a 92 amino acid L1 ORF segment was generated with the computer program CLUSTAL W. Asterisks denote sequence shift to get the best fit alignment. Dashes denote identical amino acids.

TABLE I. Evolutionary Distances Between the Most Significant and the Closest Pairs of Papillomaviruses*

Sequence compared	Distance %	Sequence compared	Distance %
HPV-X vs HPV-23	16,42	HPV-X vs HPV-15	8,96
HPV-Y vs HPV-23	11,94	HPV-Y vs HPV-15	10,45
HPV-Z vs HPV-23	11,94	HPV-Z vs HPV-15	10,45
HPV-23TRI vs HPV-23	0	HPV-23TRI vs HPV-15	11,94
HPV-X vs HPV-17	13,43	HPV-X vs HPV-37	8,96
HPV-Y vs HPV-17	8,96	HPV-Y vs HPV-37	2,99
HPV-Z vs HPV-17	8,96	HPV-Z vs HPV-37	2,99
HPV-23TRI vs HPV-17	11,94	HPV-23TRI vs HPV-37	13,43
HPV-X vs HPV-Y	10,45	HPV-Y vs HPV-Z	0
HPV-X vs HPV-Z	10,45	HPV-Y vs HPV-23T	11,94
HPV-X vs HPV-23T	16,42	HPV-Z vs HPV-23T	11,94

*The distances were calculated for amino acid position 25 to amino acid position 92 regarding to the numbering in Figure 4.

[1989], for the detection of a broad spectrum of genital HPV types. In view of the fact that the primers are strongly degenerated and that the calculated annealing temperature ranged between 53°C and 59°C the used annealing temperature applied, a critical factor in each PCR assay, must be optimized. This is important especially in view of the great numbers of HPV types, novel types, subtypes and variants which are assumed to be detected by using the primer pair MY 11/09.

Sequencing of the eleven amplified fragments showed that the eleven fragments belonged to five different HPV sequences: HPV-23TRI, HPV-X, HPV-Y, HPV-Z, and HPV-6 as shown in Figure 3. Analysis of the deduced amino acid sequences showed that all fragments display the typical feature of the partial L1 segment. As described by Bernard et al. [1994], for amino acid sequences that are encoded by the MY 11/09 segment, the sequence starts with a short stretch of conserved amino acid residues, followed by a region of great sequence diversity containing a lot of insertions and/or deletions. The remaining amino acid stretch shows strict amino acid conservation. As shown in earlier studies [Chan et al., 1992, 1995], the conserved part of the L1 fragment can be used to study the phylogenetic relationship between HPV types, subtypes and variants. Comparison of the conserved part of the detected sequences with those of known HPV types, especially with EV-associated HPV types, shows that the sequences are identical or very closely related to HPV-23, -37, or -15.

Since all amplifications with the primer pair CP65/70, which were designed specifically for EV-related HPVs, were negative a comparative analysis of the primer sequences and the new sequences was conducted. MY 11/CP65 and MY 09/CP70 correspond roughly to the same genome regions, but the CP primers are more degenerated than the MY primers. The most important difference between MY 11/09 and CP65/70 can be observed at the 3′end of the primers. Whereas the 3′end of MY 11 stops with -TGG, the 3′end of CP65 is 3 nucleotides longer and contains a degenerate position (-TGG [C/T] AT). A degenerate position is also introduced into the 3′end sequence of primer CP70. As described by Sommer and Tautz [1989] primers requires at least three homologous nucleotides at their 3′end for successful priming. In several cases with only two homologous nucleotides, no amplification could be achieved. It is possible that the degenerate positions at the 3′ends of the primers CP65 and CP70 cause considerably less effective priming of the already modified HPV sequences in trichilemmomas.

Comparison of the complete sequenced MY 11/09 DNA fragments with known HPV types, showed that all new detected sequences are clearly distinct from known types within the highly variable 5′end. The extensive deletions and/or insertions within the 5′end part of the detected sequences suggest that they could, presumably, represent putatively novel HPV types.

The finding of potentially novel HPV types is not unusual. Several groups have found untypable HPV sequences. Shamanin et al. [1994] showed that 62% of biopsies from skin lesions of 46 renal allograft patients contained DNA of known HPV DNA sequences as well as HPV sequences related to a number of EV-associated HPV types; in squamous cell carcinomas (three of 20), in dysplastic warts (three of 32), and in common warts they found a novel fragment which shares 82.7% homology to HPV-29. Also Bernard and co-workers [1994] amplified 10 distinct PCR products which could not be identified as one of the known HPV types. Berkhout et al. [1995] found six unknown sequences in a total of 12 squamous cell carcinomas. Comparison with HPV one to 67 showed that these types were closely related to EV HPVs.

According to the definition that a HPV genome represents a new HPV type when it displays more than 10% dissimilarity in the combined nucleotide sequence of the E6, E7, and L1 genes, as compared with those of any previously known one [deVilliers, 1994], isolation of the complete genome of the detected HPVs is necessary in order to verify our suggestion that they are putatively novel HPV types.

In summary, the HPV infection demonstrated in trichilemmomas and the morphological pattern of a virus induced wart suggest trichilemmoma to be a new HPV-induced epithelial hyperplasia and not a hair follicle neoplasm. This data could only be obtained by modi-

fication of PCR conditions. We believe that other morphological wart like tumours may also contain HPV DNA, detectable by the PCR protocol and sequencing of amplification products described.

REFERENCES

Ackermann AB, Wade TR (1980): Tricholemmoma. American Journal of Dermatopathology 2:207–223.

Bernard HU, Chan SY, Manos MM, Ong CK, Villa LL, Delius H, Peyton CL, Bauer HM, Wheeler CM (1994): Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphismus, nucleotide sequence, and phylogenetic algorithms. Journal of Infectious Diseases 170:1077–1085.

Berkhout RJM, Tieben LM, Smits HL, Bavinck JNB, Vermeer BJ, ter Schegget J (1995): Nested PCR approach for detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients. Journal of Clinical Microbiology 33:690–695.

Chan SY, Bernard HU, Ong CK, Chan SP, Hofmann B, Delius H (1992): Phylogenetic analysis of 48 papillomavirus types and 28 subtypes and variants: A showcase for the molecular evolution of DNA viruses. Journal of Virology 66:5714–5725.

Chan SY, Delius H, Halpern AL, Bernard HU (1995): Analysis of genomic sequences of 95 papillomavirus types: Uniting typing, phylogeny, and taxonomy. Journal of Virology 69:3074–3083.

Dyall-Smith D, Trowell H, Mark A, Dyall-Smith M (1991): Cutaneous squamous cell carcinomas and papillomaviruses in renal transplant recipients: A clinical and molecular biological study. Journal of Dermatological Sciences 2:139–146.

de Villiers EM (1989): Heterogeneity of the human papillomavirus group. Journal of Virology 63:4898–4903.

de Villiers EM (1994): Human pathogenic papillomavirus types: an update. Current Topics of Microbiology and Immunology 186:1–12.

Headington JT, French AJ (1962): Primary neoplasms of the hair follicle. Histogenesis and classification. Archives of Dermatology 86: 430–441.

Kremsdorf D, Favre M, Jablonska S, Obalek S, Rueda LA, Lutzner MA, Blanchet-Bardon C, van Voorst Vader PC, Orth G (1984): Molecular cloning and characterization of the genomes of nine newly recognized human papillomavirus types associated with epidermodysplasia verruciformis. Journal of Virology 52: 1013–1018.

Leonardi CL, Zhu WY, Kinsey WH, Penneys NS (1990): Trichilemmomas are not associated with human papillomavirus DNA. Journal of Cutaneous Pathology 13:193–197.

Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM (1989): The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells 7:209–214.

Marchuk D, Drumm M, Saulino A, Collins FS (1990): Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucleic Acids Research 19:1154.

Penneys NS, Mogollon RJ, Nadji M, Gould E (1984): Papillomavirus common antigens. Archives of Dermatology 120:859–861.

Saiki RK, Chano CA, Levenson CH, Warren TC, Boehm CD, Kazazian HH, Ehrlich HA (1988): Diagnosis of sickle cell anemia and β-thalassemia with enzymatically amplified DNA and nonradioactive allele-specific oligonucleotide probes. New England Journal of Medicine 319:537–541.

Sambrook J, Fritsch EF, Maniatis T (1992): "Molecular Cloning: A Laboratory Manual, 2nd Ed." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Schaller J, Rohwedder A, Fuchs M, Maron A, Kunze J (1996): HPV-5-Typisierung mittels nested PCR and Sequenzierung bei einer Epidermodysplasia verruciformis. Der Hautarzt 47:454–458.

Shamanin V, Glover M, Rausch C, Proby C, Leigh IM, zur Hausen H, de Villiers EM (1994): Specific types of human papillomavirus found in benign proliferations and carcinomas of the skin in immunosuppressed patients. Cancer Research 54:4610–4613.

Snijders PJF, van den Brule AJC, Schrijnemakers HFJ, Snow G, Meijer CJLM, Walboomers JMM (1990): The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. Journal of General Virology 71:173–181.

Sommer R, Tautz D (1989): Minimal homology requirements for PCR primers. Nucleic Acids Research 17:6749.

Tieben LM, ter Schegget J, Minnaar RP, Bouwes Banvinck JN, Berkhout RJM, Vermeer BJ, Jebbink MF, Smits HL (1993): Detection of cutaneous and genital HPV types in clinical samples by PCR using consensus primers. Journal of Virological Methods 42: 265–280.